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## A study on the conversion of carotene into vitamin A by fluorescence microphotometry

Saburo Iimori\*

\*Osaka Shoin Women's College,

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# A study on the conversion of carotene into vitamin A by fluorescence microphotometry\*

Saburo Iimori

## Abstract

For the purpose to confirm whether carotene is converted into vitamin A mainly in the intestine, fluorescence microscope observations as well as the fluorescence microphotometry for the estimation of fading state of the fluorescence were carried out on the fresh sections of the intestine, lung and liver of rats after oral administration of 11-carotene dissolved in oil, and suspended in water, and vitamin A in sesame oil as control. Yellowish-green fluorescence of carotene is similar to that of vitamin A in color but the fluorescence of vitamin A fades away very rapidly within one minute while that of carotene does not fade or fade more slowly than that of vitamin A. Observations have revealed that, contrary to expectation, the administered carotene is not so readily converted into vitamin A in the intestinal mucosa, but after passing through the intestine without conversion to vitamin A, it is transported to the mesenteric lymph vessels, portal vein, and reaches the liver. In the liver, carotene appears as fatty droplets or micronized particles in the parenchymal cell. The conversion of carotene into vitamin A could not be observed in the intestine, liver and lung in the observations made one hour after the oral administration of carotene. However, it seems that carotene dissolved in minute fat droplets may be converted into vitamin A at water phase in tissues, after dissolution of carotene in fat and micronization of the fat droplets.

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## A STUDY ON THE CONVERSION OF CAROTENE INTO VITAMIN A BY FLUORESCENCE MICROPHOTOMETRY

Saburo IIMORI

*Foodstuffs Laboratory, Faculty of Dietary Science, Osaka Shoin  
Women's College, Higashi Osaka, Japan*

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It is generally believed that the conversion of carotene into vitamin A in animal body takes place mainly in the intestine and some in other organs (1-10). However, looking scrutinizingly at reports concerned, there seem to be several problems to be solved before a conclusion is settled.

For example, there are some papers deducing the very conclusion solely from the histochemical methods by which carotene, vitamin A and their intermediate products are hardly distinguished from each other. In some studies the detection of vitamin A was made on the basis that the fluorescence of vitamin A alcohol disappears by about 10 minutes after UV irradiation and that of vitamin A ester by 20 minutes irradiation, asserting the conversion of caroten into vitamin A in the intestine (9, 10), but in my observation with fluorescence microscope the fluorescence of vitamin A standard oil disappears within 30 seconds by UV irradiation. Therefore, the yellowish-green fluorescence remaining longer than one minute should not be the fluorescence of vitamin A but possibly that of carotene which is administered to the animals in experiment.

Another concept in support of the theory of the intestinal conversion of carotene into vitamin A is that the parenterally administered carotene has less vitamin A-effect than that administered orally. But this fact does not necessarily indicate the main location of vitamin A formation from carotene to be intestine, because carotene given orally may be accumulated in liver and later be converted into vitamin A effectively, and the carotene given parenterally may be mainly transported into many tissues without being absorbed in fat in the intestinal lymph vessels.

Such a possible difference in distribution may be responsible for the difference in the results. Thus at present it is quite uncertain whether the conversion of carotene into vitamin A takes place mainly in intestine. For the purpose to solve this problem, I conducted a series of experiments and succeeded in the establishment of an accurate method by which carotene is distinguished from vitamin A (11-13). By using this method it has

been demonstrated clearly that in rats, most of the carotene orally administered reaches the liver in a free state or absorbed in fat when passing through the intestine and without being converted into vitamin A (14). This paper describes the findings obtained by the fluorescence method of my own design.

#### MATERIALS AND METHODS

Animals used were SD strain rats, 18 males, weighing about 170 g. All the animals were fed on only polished rice and water for about 90 days, and then they were divided into 6 groups, 3 animals in each. Group 1: untreated controls. Group 2: given oral administration of 1 ml of vitamin A oil standard product (containing  $100 \times 10^2$  I. U. of *all-trans* vitamin A acetate per g) with purified sesame oil (0.1 ml/ml), and sacrificed one hour later. Group 3: given orally 1 ml of  $\beta$ -carotene suspended in distilled water (1 mg/ml) and sacrificed one hour later. Group 4: given orally 1 ml of  $\beta$ -carotene suspensoid (1 mg/ml) as in Group 3 but sacrificed 5 hours later.  $\beta$ -carotene suspensoid was administered within 5 hours after preparation, because the fluorescence intensity and the sensitivity change with lapse of time. Group 5: given orally 1 ml of  $\beta$ -carotene dissolved in Cargille's oil (1 mg/ml) and sacrificed 1 hour later, and Group 6: given orally 1 ml of  $\beta$ -carotene solubilized in water with 15% Tween 20 (1 mg/ml) and sacrificed 1 hour later.

For the preparation of aqueous carotene solution,  $\beta$ -carotene dissolved in benzen and was added to Tween 20 solution and then homogenized by glass homogenizer. The oil solution of carotene was prepared by mixing carotene crystals simply with oil.

Prior to the oral administration of vitamin A or carotene all rats were fasted 24 hours. After sacrifice the digestive tract was immediately taken out and tissue pieces were obtained from the areas 15, 35, and 55 cm below the pylorus. These pieces of small intestine were sectioned in the length of about 8 mm, the internal contents were removed, pasted on a potato cube, and thin frozen tissue sections of about  $50\mu$  in thickness were prepared. These were washed with saline, taken on a slide glass, mounted with saline, covered with cover slip, and observed under fluorescence microscope. Similarly, the liver, lung and mesenteric lymph vessels were also observed.

For fluorescence microscopic examination, each unstained section was observed by visible light with the attached tungsten light source (S) to confirm the morphologic structure of tissue, and then the fluorescence of tissue excited by the light of the ultra high pressure mercuric lamp were observed. The specific character of the filter for the excitation by the ultra violet light is  $365m\mu$  (range: about  $300m\mu$ – $400m\mu$ ).

The intensity of fluorescence was measured on the areas of mucosal villi, submucous tissue and serous membrane at 5-second intervals. The identification of carotene with Tween and vitamin A in oil was made by the fading rate of fluorescence and other experimental conditions were described in Table 1.

TABLE 1 IDENTIFICATION OF CAROTENE, CAROTENE-FAT DROPLET, AND VITAMIN A-FAT DROPLET

|   | Light source | Carotene crystal suspended in water   | carotene-fat droplet                                      | Vitamin A-fat droplet   |
|---|--------------|---|---|---|
| Microscopy (dark field illumination)              | S            | irregular, granular in shape; dark red, orange or yellow in color.              | The inner part of the droplet is of a light yellow color. | The inner part of the droplet is dark. The margin is of a bright ringed, yellow color.      |
| Fluorescence microscopy (dark field illumination) | UV           | irregular, granular in shape; somber red, dark green, green or yellow in color. | Yellowish-green or green fluorescence fades slowly.       | Vitamin A in the droplet emits a yellowish-green or green fluorescence which fades quickly. |
|   | BV or BV+S   | irregular, granular purplish pink, pink, or bright pink.                        | The inner part of the droplet emits a light pink color.   | The inner part of the droplet is dark. The margin is of a purplish pink or pink color.      |

S: visible light from tungsten lamp

UV: ultraviolet ray from mercury lamp

BV: blue violet ray from mercury lamp through a filter for blue light

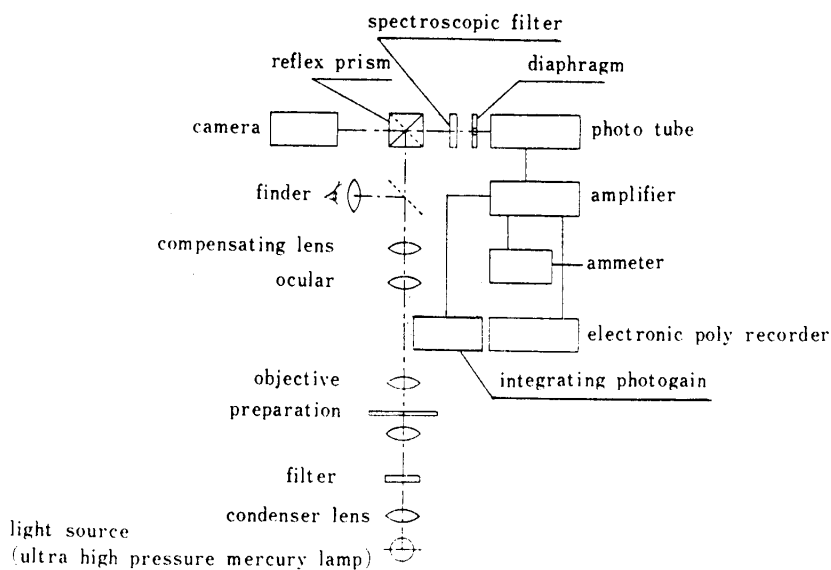


Fig. 1 The optical system of a fluorescence microphotometer

Films used were TRI-X (ASA, 400) and Recording Film (ASA, 1000). Photographs were taken at different intervals. The exposure time was within 25 seconds excepting carotene granules. The films were developed by the method of enhanced sensitivity. For measurement of fluorescence the apparatus was previously arranged to give a fixed value of fluorescence of Uranitic glass by which the

intensity of the light source was adjusted to a constant one through all the experiments. The aperture of diaphragm acting before the phototube was adjusted to 4mm in diameter, so that the area of the measurement of fluorescence intensity was kept at  $40^2 \pi \mu$  by the use of objective  $\times 20$  and ocular  $\times 5$ .

Time-lapse change or fading of the fluorescence was measured at the mucosal villi, submucosa, serous membrane of each section of digestive tract, and also of the liver and lung tissues, and the mean value from 3 rats in each group was taken as the value for each site and tissue.

Vitamin A standard oil product was purchased from the National Institute of Health, Japan and  $\beta$ -carotene, crystallized one, a synthetic product from Merck. One drop of each sample was put on an object glass 1 mm in thickness, covered with a cover slide and observed under fluorescence microscope by visible light (S), BV (blue violet) light and then UV light. The fading tendency of fluorescence of each sample was measured with a microphotometer at 5-seconds intervals for a minute from the initiation of UV irradiation by the method just described. The microphotometer used was a product of Tokyo Electronic Industrial Research Laboratory and it was attached to a Nikon Fluorescence Microscope.

## RESULTS

Through the observations on vitamin standard oil sample and carotene oil solution, it has been demonstrated that under fluorescence microscope even a minute droplet of vitamin A can be identified from that of carotene by the emission of a remarkable yellowish green fluorescence and its rapid extinction within one minute. Carotene crystal granules suspended in water present a variety of fluorescence colors, *i. e.* they may appear orange-red, yellow, yellowish green and green according to the degree of water absorption and the size of granules, and the fluorescence fades more slowly than that of vitamin A. The inherent yellowish green fluorescence of carotene in oil droplet also faded with the lapse of time, but its fading occurred very slowly. The solution of carotene in water with Tween 20 gave a remarkable yellowish green or green fluorescence and yet it faded fairly rapidly which was comparable to that of vitamin A. That is, carotene dissolved in water containing Tween is hardly distinguishable from vitamin A by fluorescence microscopy. Oil droplets, the solvent for vitamin A or carotene, also gave some fluorescence. Therefore, the fluorescence of oil droplet containing vitamin A faded very rapidly at first but the fluorescence did not disappear completely. Thus the remnant fluorescence was identified as that of oil.

On the basis of these *in vitro* observations the transition of carotene into vitamin A in the intestine and in the liver as well as in the lung was

examined after oral administration of carotene, and the following results were obtained.

In Group 1, untreated control, the liver and lung showed a rapidly fading yellowish green fluorescence, indicating the presence of vitamin A in a considerable quantity (Fig. 2). The fluorescence intensity of vitamin A in liver was higher than that of lung. In liver all the tissues including parenchymal cells gave a marked fluorescence, but in lung the fluorescence

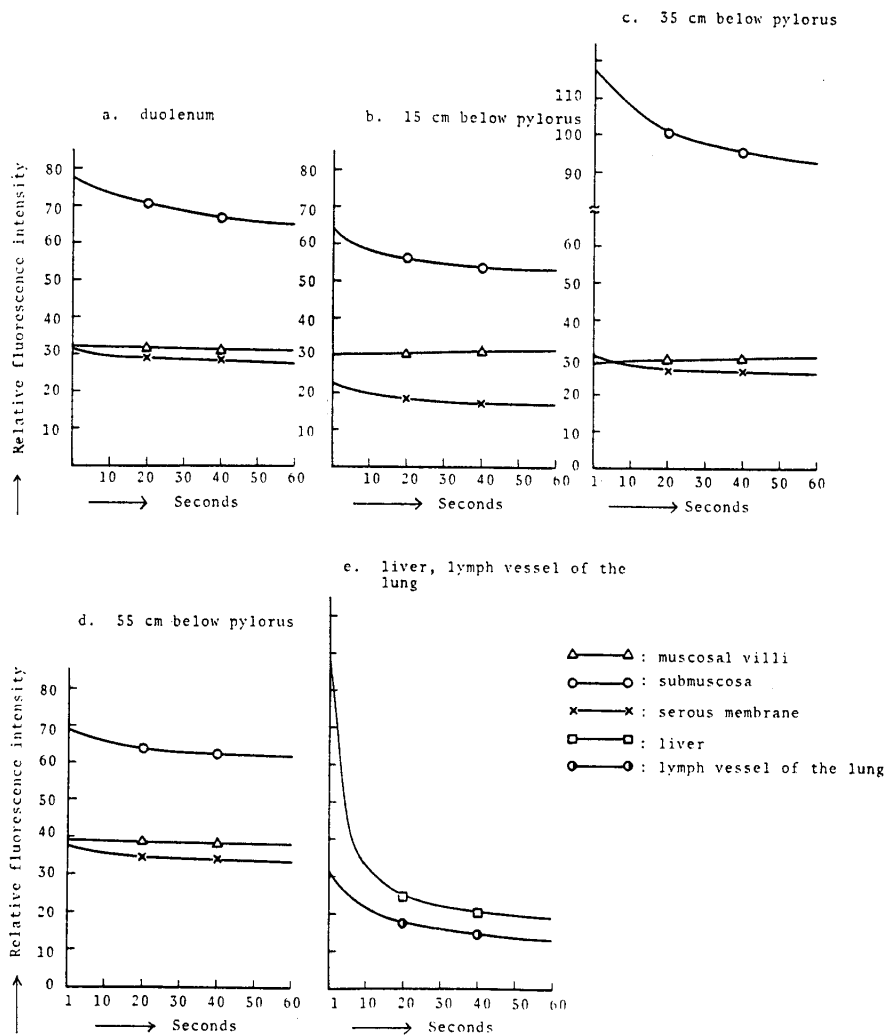


Fig. 2 Fluorescence intensity of the digestive tract at various positions, lung and liver in the untreated control rats (Group 1)

also appeared along the blood vessels and probably lymph vessels. The liver contained comparatively large vitamin A fluorescent particles, but the lung contained small vitamin A fluorescent particles of about  $4\mu$  in size (Photos 1, 2, 3). In contrast, the intestinal tissues of these animals hardly showed any readily fading fluorescence of vitamin A (Fig. 2).

In Group 2, the rats administered with vitamin A standard oil solution and sacrificed one hour later, the intensity of the rapidly fading

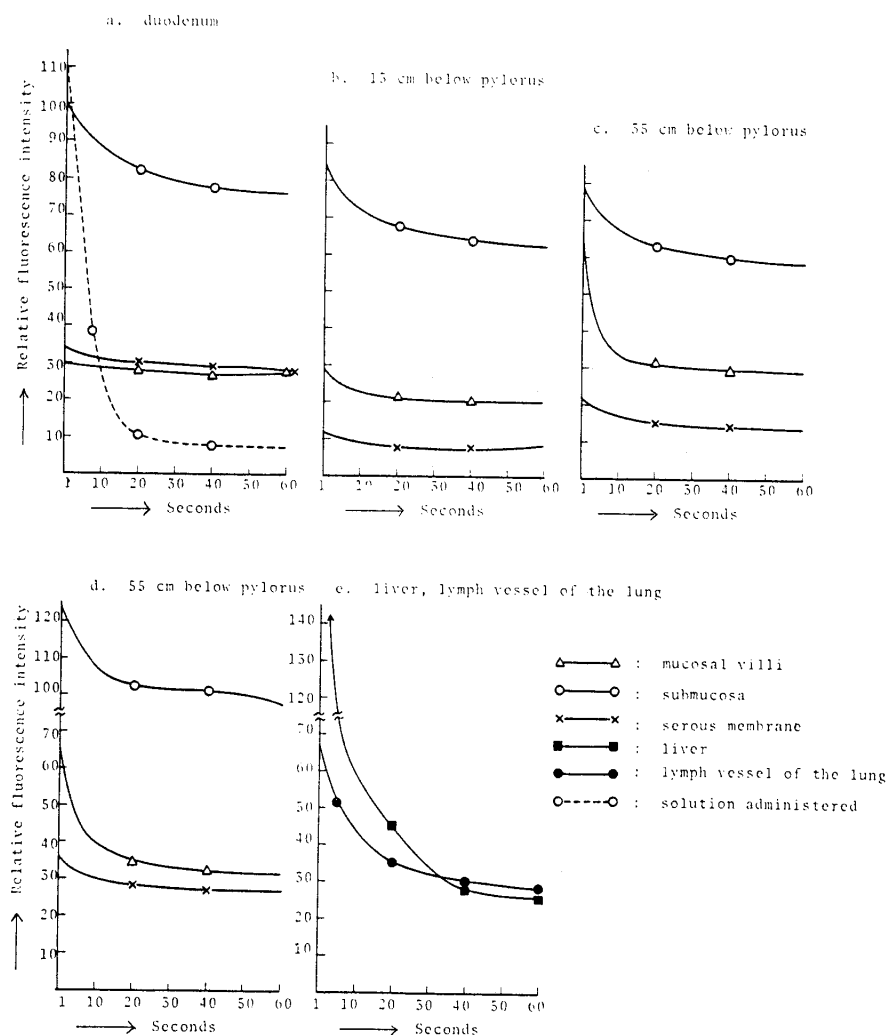


Fig. 3 Fluorescence intensity of the digestive tract at various positions, liver and lung in the rats (Group 2) one hour after oral administration of 1 ml vitamin A oil standard product with purified sesame oil (0.1 ml/ml)



fluorescence of the liver and the lung was marked as compared with that of untreated controls. In the intestinal mucosa likewise a marked readily fading fluorescence was observed (Fig. 3, c, d). In the submucosa the intensity of the readily fading fluorescence was greater than that of the untreated control, but the fading rate of the fluorescence was less than that of the intestinal mucosa and the remnant fluorescence was marked (Fig. 3).

Morphologically, the intestinal villi, the parenchymal cells of liver and lung lymph vessels showed a dense distribution of vitamin A particles that emitted a distinct yellowish green fluorescence which disappeared rapidly.

The submucosa gave a distinct fluorescence as marked as in the mucosal villi, but vitamin A fluorescence could hardly be detected being interfered by a strong fluorescence of the stroma. In the mucosal villi a number of the yellowish green fluorescent particles were observed, whose fluorescence faded away rapidly but some remnant fluorescence was also observed. Of such fluorescent particles the faded ones would be vitamin A particles and the remnant fluorescence would be due to some other fluorescent substances contained in diet or those in sesame oil, the solvent of vitamin A standard solution (Photos, 4, 5).

In Groups 3 and 4, the animals were sacrificed one hour and 5 hours, respectively after the administration of  $\beta$ -carotene suspended in water. The observations in both groups revealed faint yellowish green fluorescence in the mucosa and serous membrane of small intestine. Generally such fluorescence tended to slightly decrease in intensity with the lapse of time, while in some areas the intensity was slightly increased (Figs. 4, 5). The decrease in the fluorescence intensity of submucosa was less than that of the submucosa of controls. The liver showed a moderate yellowish green fluorescence, but it was extremely minimal comparing to the liver of untreated controls.

Morphological observations by UV, BV and S revealed that the mucosal villi and serosa contained densely distributed carotene particles. The fluorescence of these particles faded slowly with the lapse of time. The fading grade of fluorescence was somewhat inferior to carotene water suspensoid. Fluorescent particles, whose fading rate was comparable to that of vitamin A, in this instance was not observed (Photos, 6, 7). In the intestinal submucosa the stroma of tissue gave a strong fluorescence with UV, and the fluorescent particles of vitamin A and carotene were hardly detectable. The lymph vessels of mesentery also showed yellowish green fluorescence whose fading rate was comparable to that of free carotene.

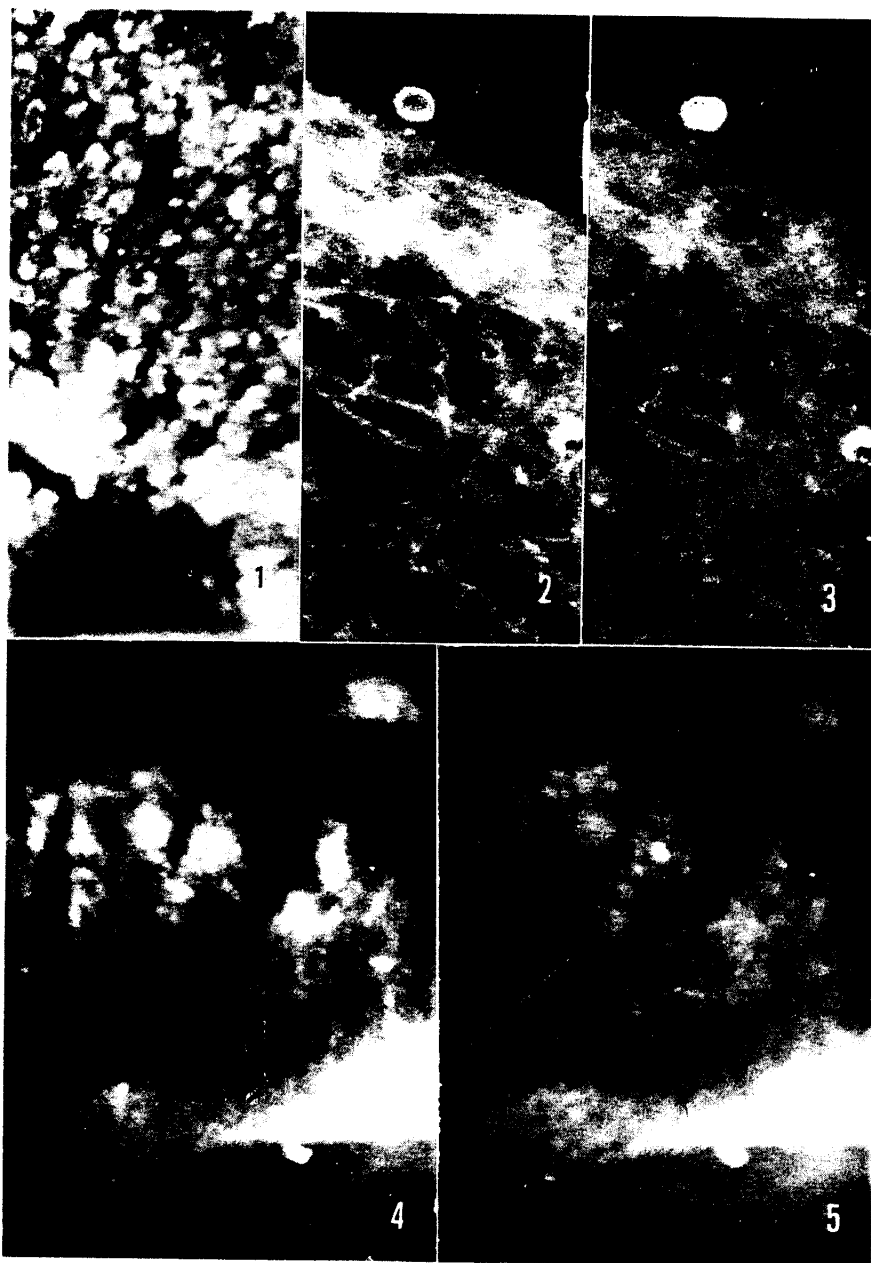


Photo. 1 Fluorescence of the liver of untreated control fed on polished rice for about 90 days as other test rats. Diffuse, granular white spots on the tissue represent the fluorescence of vitamin A. Exposed for 15 sec, under UV, illumination.  $\times 120$

Photo. 2 Picture of fluorescence of vitamin A of the lymph vessel in the lung of a control, exposed for 15 sec.  $\times 120$

# Conversion of Carotene into Vitamin A

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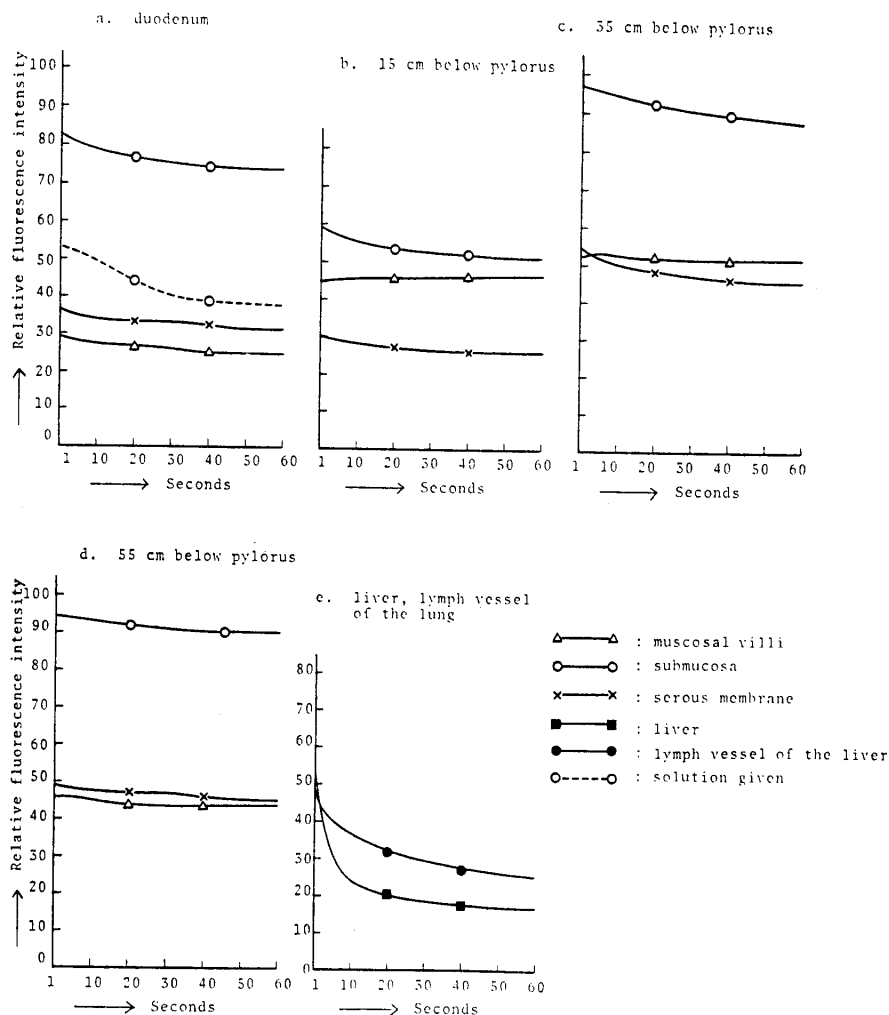


Fig. 4 Fluorescence intensity of the digestive tract at various positions, liver and lung of the rats (Group 3) one hour after oral administration of 1 ml of  $\beta$ -carotene suspended in distilled water (1 mg/ml)

Photo. 3 Picture of the fluorescence of the identical tissue as in Photo. 2, but taken after 10 sec. Fluorescence of vitamin A has faded away but that of non-vitamin A remains. Exposed for 15 sec.  $\times 120$

Photo. 4 Fluorescence of vitamin A of the rat intestine 35cm below the pylorus. The picture was taken 1 hour after the oral administration of 1 ml vitamin A oil standard product with purified sesame oil (0.1 ml/ml).  $\times 120$

White diffuse region appearing on the mucosal villi represents the fluorescence of vitamin A. Exposed for 20 sec.  $\times 120$

Photo. 5 Picture of fluorescence of the same tissue as in Photo. 4, but taken 10 sec. later. The vitamin A fluorescence of the villi has faded out. Exposed for 20 sec.  $\times 120$

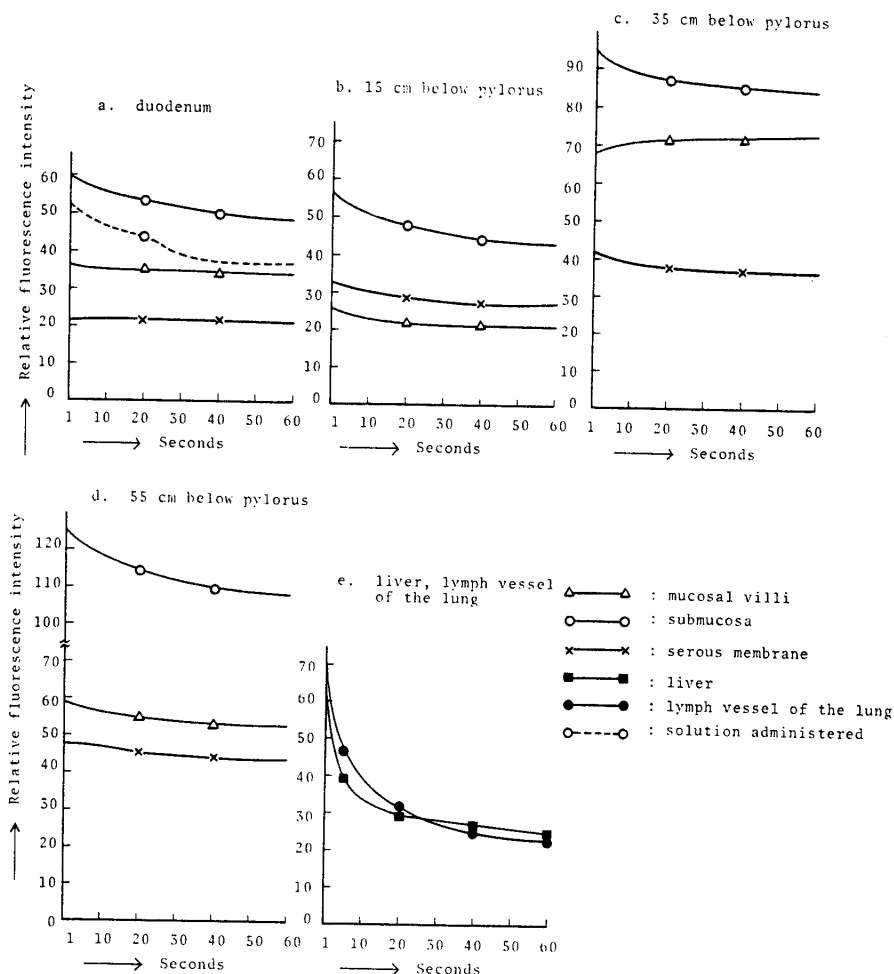


Fig. 5 Fluorescence intensity of the digestive tract at various positions, liver and lung of the rats (Group 4) 5 hours after administration of 1 ml of  $\beta$ -carotene suspended in distilled water (1 mg/ml)

Under high magnification the lymph vessels revealed carotene oil droplets, emitting yellowish green fluorescence (Photo 8).

In Group 5, where the animals were given  $\beta$ -carotene dissolved in Cargille's oil orally and sacrificed one hour later, the intestine showed a distinct yellowish green fluorescence. The fluorescence faded very slowly and the fading tendency was less than that of carotene oil solution (Fig. 6), (Photos, 9, 10). The rapidly fading fluorescence characteristic of vitamin A was hardly observed in any part of the intestine. In some parts

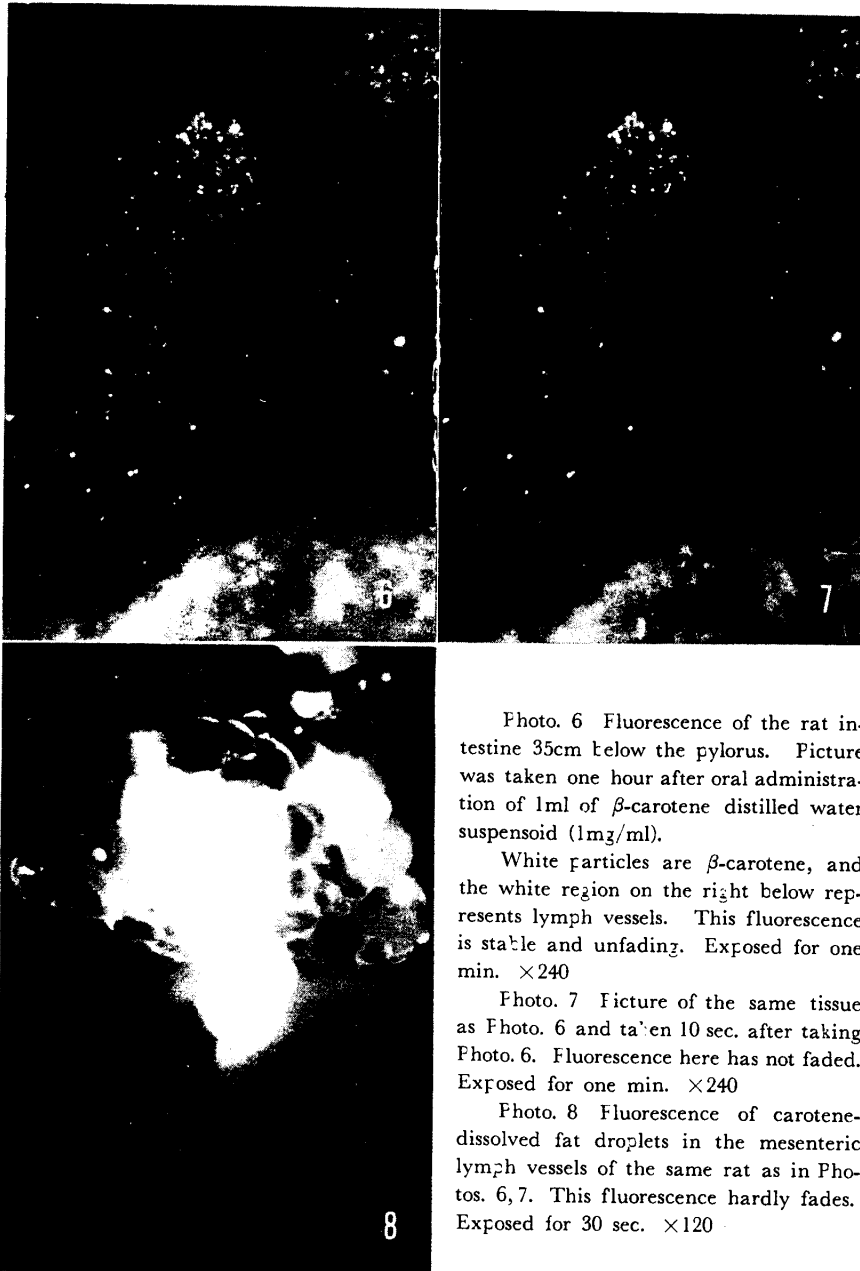


Photo. 6 Fluorescence of the rat intestine 35cm below the pylorus. Picture was taken one hour after oral administration of 1ml of  $\beta$ -carotene distilled water suspensoid (1mg/ml).

White particles are  $\beta$ -carotene, and the white region on the right below represents lymph vessels. This fluorescence is stable and unfading. Exposed for one min.  $\times 240$

Photo. 7 Picture of the same tissue as Photo. 6 and taken 10 sec. after taking Photo. 6. Fluorescence here has not faded. Exposed for one min.  $\times 240$

Photo. 8 Fluorescence of carotene-dissolved fat droplets in the mesenteric lymph vessels of the same rat as in Photos. 6, 7. This fluorescence hardly fades. Exposed for 30 sec.  $\times 120$

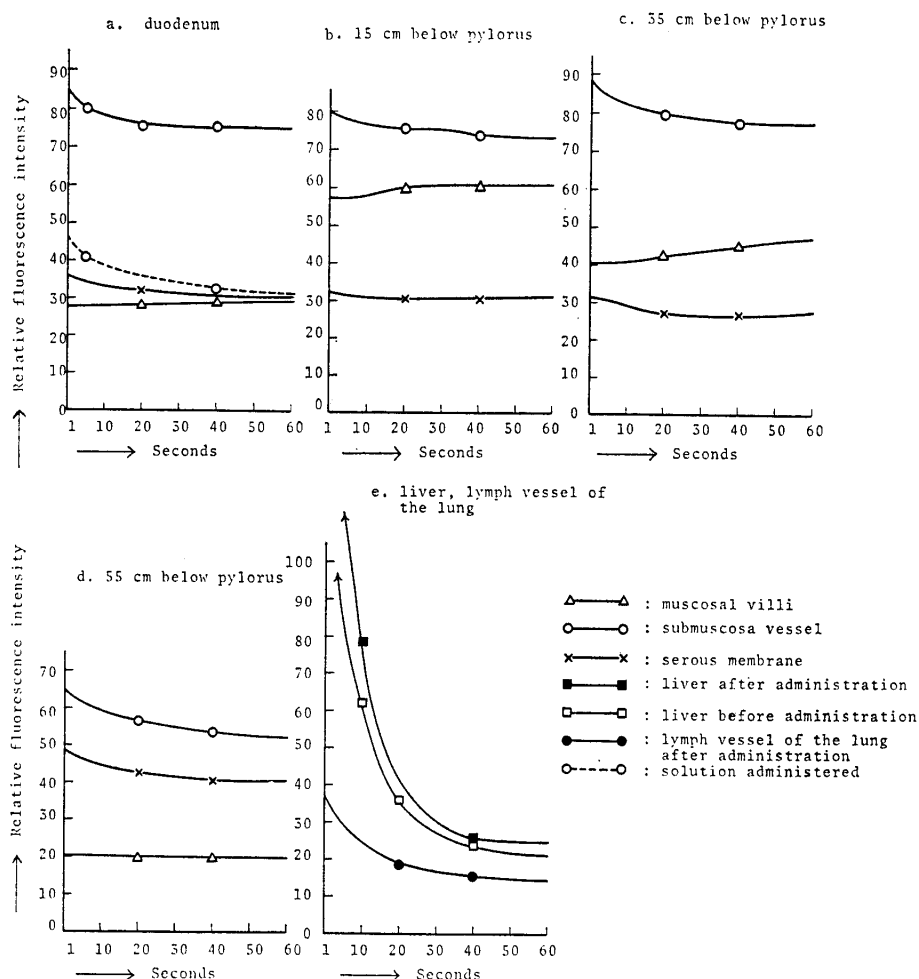


Fig. 6 Fluorescence intensity of the digestive tract at various positions, liver and lung in the rats (Group 5) one hour after oral administration of 1 ml of  $\beta$ -carotene dissolved in Cargille's oil (1 mg/ml)

the intensity of fluorescence appeared to be rather increased by the continued UV irradiation.

The liver one hour after the oral administration of carotene-oil solution showed a strong yellowish green fluorescence whose intensity was more marked than that of the liver partially detected before the carotene administration. The fading was rapid, but the fading rate and the residual fluorescence were nearly the same in both livers showing no tendency of enhancement of vitamin A synthesis (Fig. 6-e) (Table 2).

TABLE 2. FADING OF FLUORESCENCE INTENSITY IN RAT LIVER BEFORE AND AFTER ORAL ADMINISTRATION OF  $\beta$ -CAROTENE-CARGILLE'S OIL SOLUTION (1mg/ml), 1ml PER ANIMAL

| Fluorescence intensity                                 | Fading state of fluorescence (second) |     |    |    |    |    |    |    |    |    |    |    |    |       | Integrated value |
|--|---------------------------------------|-----|----|----|----|----|----|----|----|----|----|----|----|-------|------------------|
| seconds  | 1                                     | 5   | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | Total |                  |
| Fluorescence intensity                                 |                                       |     |    |    |    |    |    |    |    |    |    |    |    |       |                  |
| (A) value before carotene administration               | 140                                   | 88  | 63 | 45 | 39 | 32 | 28 | 25 | 23 | 20 | 19 | 19 | 19 | 560   |                  |
| (A') values after subtracting remnant fluorescence     | 121                                   | 69  | 44 | 26 | 20 | 13 | 9  | 6  | 4  | 1  | 0  | 0  | 0  | 313   |                  |
| (B) values one hour after administration               | 160                                   | 105 | 79 | 55 | 42 | 33 | 29 | 26 | 25 | 24 | 23 | 23 | 23 | 647   |                  |
| (B') values after subtracting the remnant fluorescence | 137                                   | 82  | 56 | 22 | 19 | 10 | 6  | 3  | 2  | 1  | 0  | 0  | 0  | 338   |                  |
| B'—A'  | 16                                    | 13  | 12 | —4 | —1 | —3 | —3 | —3 | —2 | 0  | 0  | 0  | 0  | 25    |                  |

The fluorescence related to vitamin A was determined by subtracting the total remnant fluorescence from the total fluorescence for one minute. Thus the total remainder value of the liver obtained 338 one hour after administration of carotene-oil solution and that before the administration was 313. The difference between the two values, 25, may indicate the newly converted vitamin A, but this value is too small to decide the increase in the rapidly fading fluorescence.

Morphological observation revealed that the fluorescence of the minute carotene particles hardly faded as rapidly as that of vitamin A. Morphologically, the intestinal mucosa contained no vitamin A fluorescence particles but distinct oil droplets of  $\beta$ -carotene which emitted a slowly fading yellowish green fluorescence (Photos, 9, 10). Thus, most of the fluorescence seen in the intestine of the animals in Group 5 seems to be of carotene oil. Morphological observations of the liver of these animals revealed a remarkable fluorescence in the parenchymal cells of liver (Photo, 11). Besides these, a number of fluorescent particles of  $\beta$ -carotene were observed in the capillaries in the liver. The slow fading character of yellowish green fluorescence of fat droplets in the liver was comparable to that of carotene-oil droplets administered to animals orally. This indicates that carotene-oil given orally reaches the liver passing through the intestinal wall without being transformed into vitamin A.

In animals of Group 6, which were administered carotene aqueous solution with Tween 20, the intestinal mucosa showed a diffuse yellowish green fluorescence that faded fairly rapidly like the fluorescence of vitamin A.

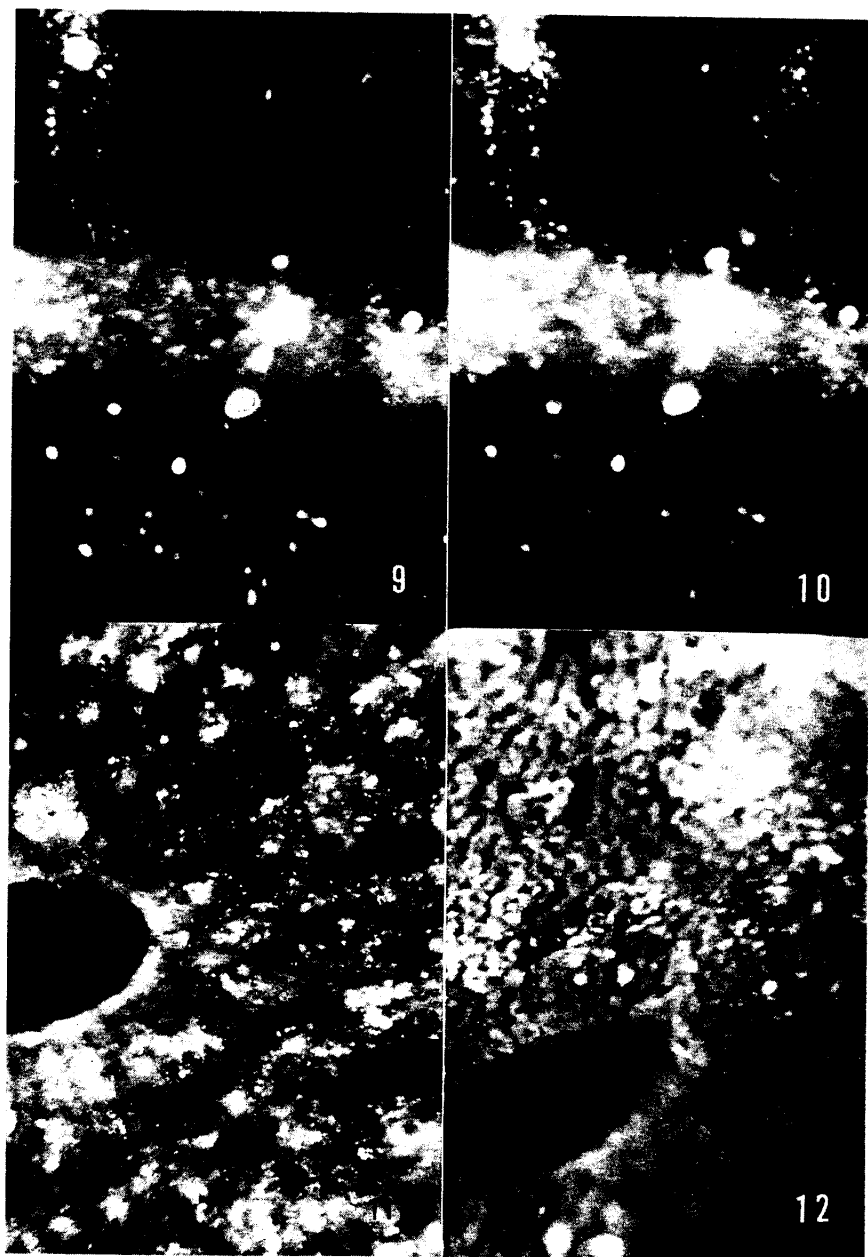


Photo. 9 Fluorescence of the rat intestine 35cm below the pylorus. The picture was taken one hour after the oral administration of 1ml  $\beta$ -carotene-Cargille's oil solution (1mg/ml). White particles represent mainly carotene-oil particles. Exposed for 30 sec.  $\times 120$

Photo. 10 Fluorescence microscope picture of the same tissue as in Photo. 9, but taken 10 sec. later, and indicates no fading of fluorescence. Exposed for 30 sec.  $\times 120$



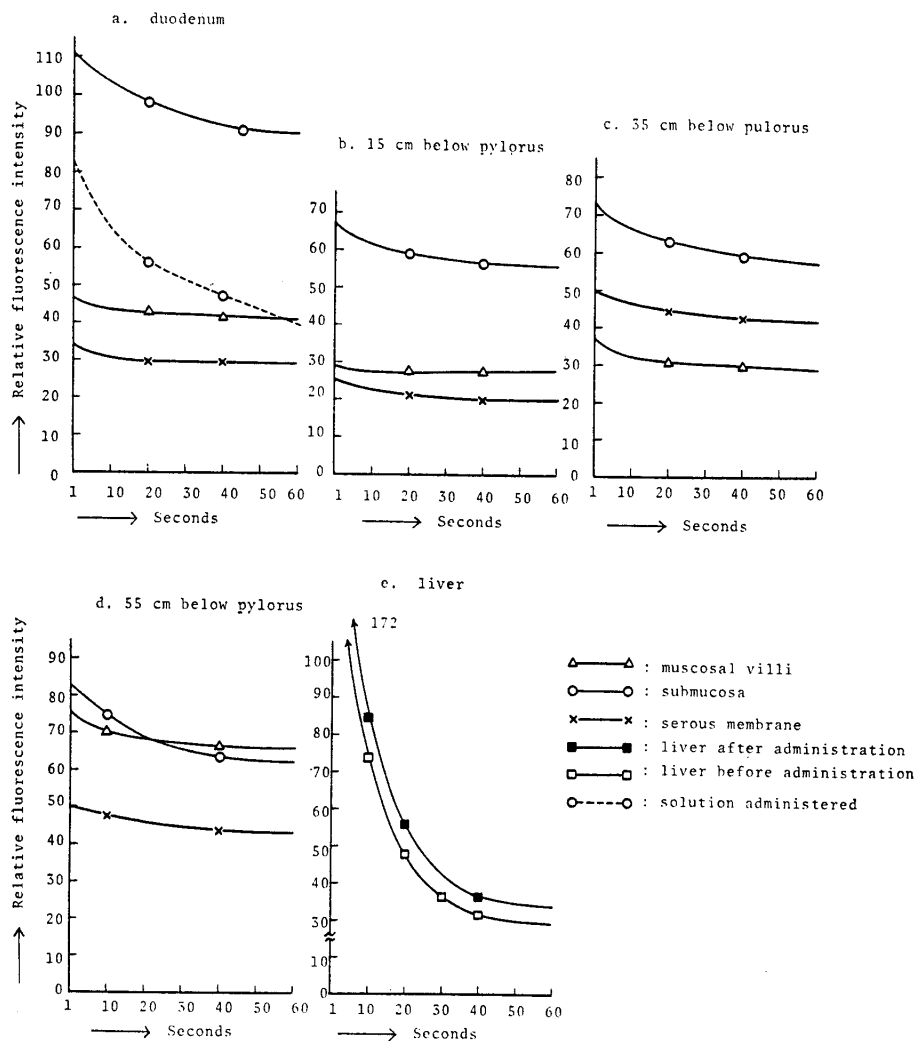


Fig. 7 Fluorescence intensity of the digestive tract at various positions liver and lung (Group 6) one hour after oral administration of 1 ml of  $\beta$ -carotene solubilized in water with 15% Tween 20 (1 mg/ml)

Photo. 11 The fluorescence microscope picture of the liver of the same rat as in Photos. 9, 10. The fluorescence appears brighter than that before carotene administration, probably due to an accumulation of  $\beta$ -carotene oil droplets in addition to the pre-existing vitamin A. Exposed for 30 sec.  $\times 120$

Photo. 12 Fluorescence of the liver one hour after the oral administration of 1 ml of  $\beta$ -carotene dissolved in 15% Tween 20 aqueous solution (1 mg/ml).  $\times 120$

However, the fluorescence of similar character was observed with the carotene aqueous solution with Tween 20 administered to the animals, indicating that the fluorescence should be of  $\beta$ -carotene-Tween mixture absorbed in the tissue of the intestinal mucosa, but not of vitamin A (Fig. 7). The liver tissue also presented a marked increase in fluorescence. The fluorescence faded relatively fast, but the morphologic picture was different from that of the liver of untreated group or of vitamin A-administered group. The fluorescence appeared rather diffuse (Photo. 12) and the morphologic picture differed from that of the liver (Photo. 11) from the animals administered carotene in Cargille's oil. These results seem to indicate that  $\beta$ -carotene-Tween 20 mixture reaches liver passing through intestine without being transformed into vitamin A.

#### DISCUSSION

Vitamin A dissolved in oil and smeared on the object glass gives a distinct yellowish green fluorescence which fades very rapidly within one minute by UV illumination as shown under fluorescence microscope.  $\beta$ -carotene dissolved in oil also shows the fluorescence similar in color, but it hardly fades or fades very slowly. Free carotene granules suspended in water give orange-red, yellow, yellowish-green or green fluorescence according to the degree of water absorption and the size of the granules, but such fluorescence fades slowly and the fading rate differs from that of vitamin A and can be distinguished from the latter. However, both Tween 20 and aqueous solutions of  $\beta$ -carotene with Tween 20 give rapidly fading, yellowish-green or green fluorescence similar to that of vitamin A. Therefore, it is difficult under fluorescence microscope to identify vitamin A from the carotene aqueous solution with Tween. Thus, the observation revealed that vitamin A and carotene aqueous solution cannot be distinguished from each other by the color of the fluorescence and by measuring the fading rate of fluorescence.

The fluorescence microphotometry method devised by myself proves to be useful in identifying vitamin A from carotene in the tissues. One hour after the oral administration of carotene suspended in water to rats a quantity of carotene particles has been observed in the intestinal mucosa, as revealed by the yellowish-green fluorescence which fades slowly. Hardly any vitamin A fluorescence is observed in the intestinal mucosa, submucosa and serosa. Observation five hours after  $\beta$ -carotene administration gives also identical results. These findings indicate clearly that the orally-administered  $\beta$ -carotene is hardly converted into vitamin A in the intestinal

wall.

The concept that carotene is converted into vitamin A in the intestine seems to be drawn erroneously on the basis of misdetecting the fluorescence of carotene or carotene dissolved in fat droplet for that of vitamin A (9, 10). If carotene is absorbed from the intestine without conversion into vitamin A, carotene in a free state or dissolved in a fat droplet should be detected in the mesenteric lymph vessels, thoracic duct and portal vein. This is true as observed clearly in these tissues of the animal sacrificed one hour after oral administration of carotene, especially that suspended in water, where small carotene crystals are seen in the vessels. One or five hours after oral administration of  $\beta$ -carotene water suspensoid, the liver of rats shows a slight decrease in the intensity of yellowish-green fluorescence which fades more slowly than the liver of the untreated controls or of the vitamin A administered animals.

The reason for this may be explained as that free carotene granules are transferred and accumulated in the liver without being converted into vitamin A and that these granules prevent the appearance of the fluorescence of the pre-existing vitamin A. In the animals given carotene dissolved in oil the liver shows a more remarkable yellowish-green fluorescence than the liver where it is partially detected before carotene oil administration. The fluorescence fades readily both before and after administration of carotene dissolved in oil, but the difference of the values in the liver after carotene administration and that before the administration is extremely small and new synthesis of vitamin A from carotene is hardly recognized. Morphologic observation reveals that the fading fluorescence like vitamin A also comes from the micronized particles of carotene or carotene oil droplets. The picture suggests the conversion of micronized carotene fat droplets into vitamin A droplets to occur in parenchymal cells of the liver after taking carotene prior to the conversion of carotene. But in the present experiment the quantitative estimation reveals hardly any increase in contents both in the intestine and liver. However, the liver will possibly be the main organ of vitamin A production, because the former experiment made on tadpole by myself indicated that the carotene administered to the tadpole was largely accumulated in the liver first and then the conversion of carotene into vitamin A occurred as revealed by fluorescence microphotometry (14). Carotene dissolved in water by the aid of Tween 20 gives a fairly strong yellowish-green fluorescence but the fading rate is nearly the same as that of vitamin A. Therefore, carotene in water-Tween mixture cannot be distinguished from vitamin A by fluorescence microscope and microphotometry and it is not suitable to use such

a solution for this sort of investigation.

#### SUMMARY

For the purpose to confirm whether carotene is converted into vitamin A mainly in the intestine, fluorescence microscope observations as well as the fluorescence microphotometry for the estimation of fading state of the fluorescence were carried out on the fresh sections of the intestine, lung and liver of rats after oral administration of  $\beta$ -carotene dissolved in oil, and suspended in water, and vitamin A in sesame oil as control.

Yellowish-green fluorescence of carotene is similar to that of vitamin A in color but the fluorescence of vitamin A fades away very rapidly within one minute while that of carotene does not fade or fade more slowly than that of vitamin A.

Observations have revealed that, contrary to expectation, the administered carotene is not so readily converted into vitamin A in the intestinal mucosa, but after passing through the intestine without conversion to vitamin A, it is transported to the mesenteric lymph vessels, portal vein, and reaches the liver.

In the liver, carotene appears as fatty droplets or micronized particles in the parenchymal cell.

The conversion of carotene into vitamin A could not be observed in the intestine, liver and lung in the observations made one hour after the oral administration of carotene.

However, it seems that carotene dissolved in minute fat droplets may be converted into vitamin A at water phase in tissues, after dissolution of carotene in fat and micronization of the fat droplets.

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